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ABSTRACT

The amounts of zinc-65 accumulated from water and from food by flounder, *Paralichthys* sp., were measured and compared. When concentration factors were calculated, it was found that flounder concentrated zinc-65 to higher levels from food than from water. The initial rate of accumulation of zinc-65 from water was dependent on the concentration of the radionuclide in the water. Results of experiments reported indicate that the transfer of zinc-65 through the food chain is a more important pathway of accumulation for fish than direct accumulation from water.

INTRODUCTION

Accumulation of zinc-65 by flounder was measured, since this radionuclide is known to be one of the more abundant neutron-induced isotopes released from nuclear reactors and since zinc is known to be an essential element in the metabolism of most animals. Zinc-65 released into the Columbia River by the Hanford Atomic Products Operation was the only reactor effluent found in sufficient abundance beyond the mouth of the river to be of radiological interest (Nelson, 1962). Also, zinc-65 has been reported to account for much of the activity in marine animals taken from the Pacific testing grounds (Lowman, Palumbo, and South, 1957; Donaldson, 1959).

In the laboratory, Chipman, Rice, and Price (1958) followed the accumulation of zinc-65 by fish from seawater and from oral doses of the isotope. In these experiments, rates of uptake and tissue distribution were determined for the croaker, *Micropogon undulatus*, and retention was followed in the pinfish, *Lagodon rhomboides*. The main tissues of accumulation in the croakers were bone, integument, and muscle. Pinfish retained a small per cent of zinc-65 for long periods of time.

Since much of the radioactivity released into rivers and estuaries is often quickly removed from the water by sediments and plankton, the most important source of radionuclides for animals may be plants and animals that have already concentrated the isotope. Davis and Foster (1958) found food to be the main source of radioactivity for fish in the Columbia River.

Laboratory experiments reported in this paper were designed (1) to follow the accumulation of zinc-65 by flounder, both from seawater and from ingested foods, and (2) to determine what effect the concentration of the isotope in the water had on the rate of accumulation. This study was an attempt to simulate in the laboratory a situation that might occur in nature if a reactor continuously released effluent into an estuary. Young flounder were chosen as experimental animals since they spend their postlarval and juvenile stages in the estuary.

MATERIALS AND METHODS

Three species of flounder, *Paralichthys dentatus*, *P. albigutta*, and *P. lethostigma*, were collected with a meter plankton net (1 mm mesh) as they migrated from the ocean spawning grounds into the estuaries near Beaufort, N. C. When collected, the fish were in the postlarval stage of development. Before being used in the experiments, they were maintained in the laboratory until they reached the juvenile stage. No attempt was made to separate the three species.

The food selected for the experiment was nauplii of the brine shrimp, *Artemia salina*, a crustacean similar in size to zooplankton eaten by juvenile flounder in their natural environment. The nauplii were maintained for 24 hours, prior to being fed to the fish, in seawater containing 0.001 microcurie per ml of zinc-65. At the end of 24 hours, 0.4 g of the nauplii was removed from the water, measured for radioactivity, and fed to the fish. The same procedure was followed in raising the nonactive nauplii, but nonactive water was used.

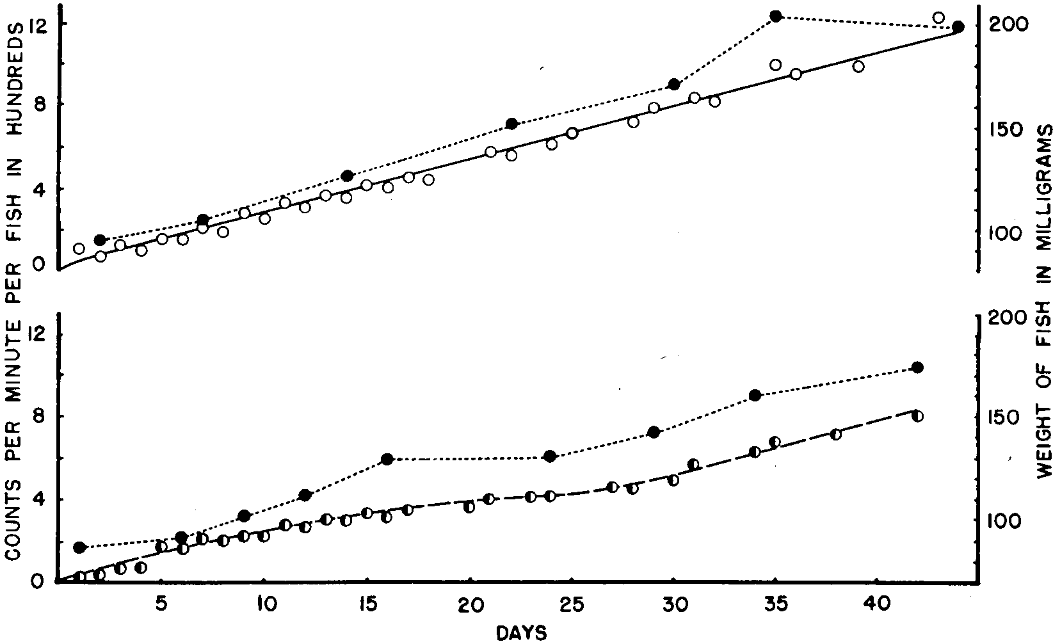

FIGURE 1.—Accumulation of zinc-65 by flounder in relation to change in weight. Closed circles represent average weight of flounder, open circles represent accumulation from water, and half-closed circles accumulation from food.

To compare possible pathways of accumulation, three groups of flounder, each containing 20 fish, were exposed to zinc-65 in the following manner:

(1) One group of fish was placed in a liter of seawater and fed 0.4 g of radioactive brine shrimp every other day. On the alternate days, the fish were returned to clean seawater. The only source of zinc-65 available to these fish was food.

(2) A second group of flounder was placed in a liter of seawater containing the same amount of activity as the water in which the labeled brine shrimp were raised. On alternate days, these fish were placed in nonactive seawater and fed 0.4 g of nonactive brine shrimp nauplii. The only source of zinc-65 for this group of fish was the radioactive water.

(3) The third group of fish was held in water with the above concentration of zine-65 every other day and on alternate days the fish were transferred to nonactive water and fed active food. Both food and water were possible pathways of accumulation of zinc-65 to this group of fish.

The effect of increasing the amount of zinc-65 in the water on the rate of zinc-65 accumulation by flounder was measured by placing one group of 10 juvenile flounder for 2 weeks in a liter of seawater containing 0.004 microcurie of zinc-65, and by placing a second group of fish for 1 week in a liter of seawater containing 0.008 microcurie of zinc-65. Nonactive food was placed in the active water daily so that some accumulation occurred from food as well as water.

Carrier-free zinc-65 with a half-life of 245 days in the chemical form of zinc chloride dissolved in acid was used in amounts which did not increase the measurable zinc concentration when added to water.

Seawater was filtered through cotton to remove particulate material. All experiments were conducted at room temperature (19° to 24° C) and the salinity ranged from 25.2 to 33.7 ppt. The water was changed every day to prevent a buildup of waste products.

The amount of zinc-65 present was measured with a gamma-ray scintillation detector and a well-type 3-inch sodium iodide crystal. Each measurement was corrected for decay

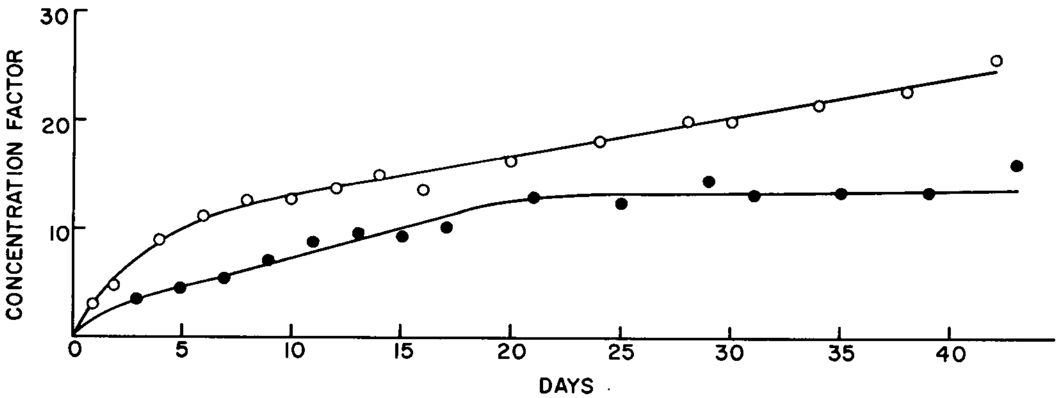

FIGURE 2.—Comparison of concentration of zinc-65 by flounder from food and water. Open circles represent concentration factor over food; closed circles represent concentration factor over water.

and background radiation. After exposure to zinc-65, each fish was removed from the tank, rinsed in nonactive water, and measured for contained activity. Radioactivity in all fish was measured at each sampling time.

ACCUMULATION FROM FOOD AND WATER

Uptake of zinc-65 by flounder from food was compared to the uptake of zinc-65 from water for a period of 44 days. Both groups originally consisted of 20 fish; however, during the experiment six fish died in the group held in radioactive water and four fish died in the group fed radioactive food.

Accumulation of zinc-65 by flounder continued throughout the experiment whether the source of activity was food or water (Figure 1). Variation in the rate of accumulation may be attributed to changes in rates of growth of the fish during the experiment. When the zinc-65 of the fish in active water was based on a concentration factor (counts per minute per gram of fish compared to counts per minute per gram of water), it was found that an apparent equilibrium with the water was reached after 20 days (Figure 2). The apparent equilibrium may be attributed to a "biological dilution" effect caused by increased weight. Baptist and Price (1962) reported that uptake of cesium-137 from seawater based on weight of flounder was reduced during periods of rapid weight increases due to the fish increasing in mass more rapidly than the isotope was accumulated.

The fish obtaining zinc-65 from their food concentrated the isotope over the amount in food throughout the experiment (Figure 2). The concentration factor (counts per minute per gram of fish compared to counts per minute per gram of food) for fish in the accumulation from food experiment was calculated on each fish eating 0.02 g of food containing 177 counts per minute. When the concentration factors for the two groups of fish were compared, it was found that fish obtaining zinc-65 from food contained 1.6 times more zinc-65 than fish obtaining zinc-65 from water. This may appear to contradict the data in Figure 1, until one realizes that the data in Figure 1 were based on the amount of radioactivity per fish, regardless of availability, and that data in Figure 2 were based on concentration factors.

Accumulation by fish having zinc-65 available from both seawater and food was followed for a period of 20 days. Under these conditions, the amount of zinc-65 accumulated was approximately equal to the sum of the amounts accumulated from food and water when these sources of zinc-65 were available individually (Figure 3).

EFFECT OF CONCENTRATION OF ZINC-65 IN WATER ON RATE OF ACCUMULATION

Accumulation of zinc-65 by juvenile flounder from water containing 0.004 and 0.008 microcurie per milliliter was measured over a 14-day period. Values were based on averages

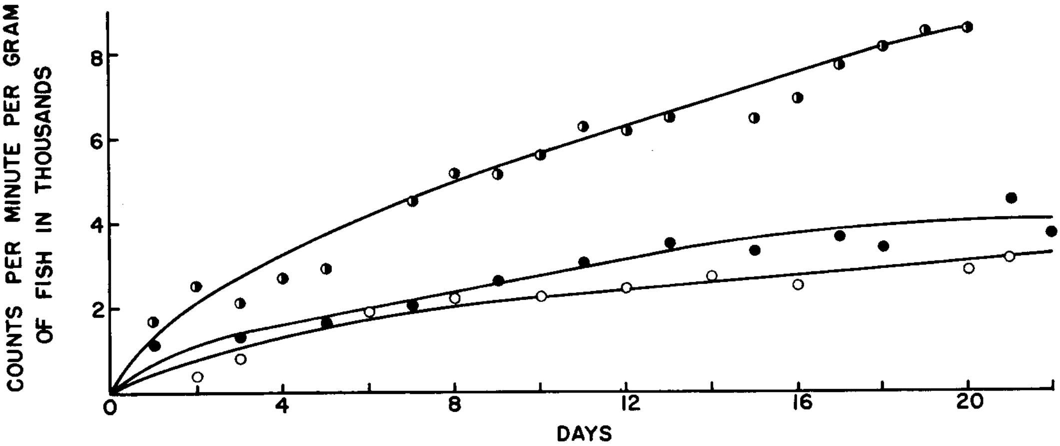

FIGURE 3.—Accumulation of zinc-65 by flounder from food, water, and a combination of both food and water. Half-closed circles represent accumulation from both food and water, closed circles represent accumulation from water, open circles accumulation from food.

of 10 fish taken at each sampling time. Standard deviations and differences between means were calculated to determine if accumulation was significantly different between the two groups at 7 and 14 days.

Doubling the amount of zinc-65 in the water increased the rate of accumulation so that fish in 0.008 microcurie per liter reached the same level of activity as fish in 0.004 microcurie per liter in one-half the time (Figure 4). The mean counts per minute per gram of fish in 0.004 microcurie per liter after 14 days were not significantly different from the mean counts per minute per gram of fish in 0.008 microcurie per liter after 7 days.

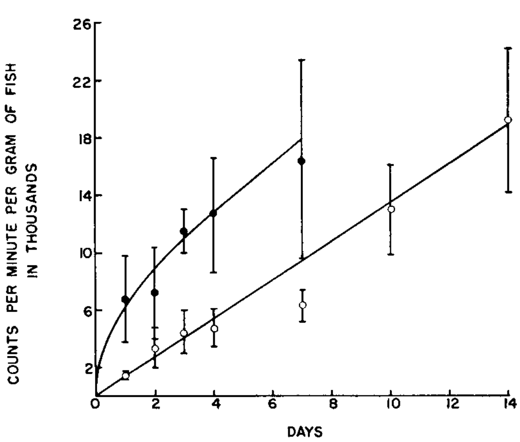

FIGURE 4.—Effect of zinc-65 concentrations in the water on its rate of uptake by flounder. Closed circles represent uptake from water containing 0.008 microcurie per ml, open circles 0.004 microcurie per ml.

DISCUSSION

In the present experiments, the accumulation of zinc-65 by flounder from food was compared to its accumulation of zinc-65 from water by separating these two pathways in the laboratory. It is relatively easy to follow the accumulation of an isotope from water; however, it is more difficult to follow the accumulation from food. The food must be one that will accumulate and retain the desired isotope; it must be readily available, and it must be an organism that the fish will eat. One method used in the past was to simulate feeding by giving the fish an oral dose or repeated oral doses of an isotope. This has an advantage in that the strength of the dose is easily controlled so that each fish is given the same amount of activity. However, a disadvantage to this method is that the accumulation from a liquid or gelatin dose may not be comparable to the accumulation from a natural food. Schiffman (1961) showed that Sr^{90}–Y^{90} was more readily accumulated by trout from gelatin than from natural foods; thus results based on the accumulation of Sr^{90}–Y^{90} by fish from gelatin may not show accurately what would occur under natural conditions.

Krumholz (1956) pointed out that the element concentrated most by the food organism may not be the element concentrated highest by the consumer organism. By conducting experiments in which a food chain is involved, a better estimation can be made of what would happen if a natural food source were to become polluted with radioactivity.

The amount of activity available to the fish in these experiments was limited by the amount of food. Ideally, the best way to conduct a feeding experiment would be to duplicate the conditions found in nature where food is available at all times. In this experiment, had it been possible to keep the optimum amount of food available to the fish at all times, the difference in concentration factors between the two groups might have been even greater.

Another factor to be considered, when comparing accumulation from food to accumulation from water, is the total amount of radioactive zinc available. Assuming that all of the zinc-65 in the water or food was available, the group of fish accumulating zinc-65 from water was exposed to 22 microcuries during the experiment, while the group of fish accumulating zinc-65 from food was exposed to 0.2 microcurie. At the end of 43 days, the fish accumulating zinc-65 from water contained a total of 0.05 microcurie or 0.2 per cent of the total amount of zinc-65 available, while fish accumulating zinc-65 from food contained a total of 0.04 microcurie or 20 per cent of the total zinc-65 available.

It was found that brine shrimp nauplii accumulated zinc-65 from water at a faster rate than flounder. From this it can be concluded that in the natural environment where all organisms would be exposed to radioactivity, the amount of zinc-65 passing through the food chain to flounder would be greater than that obtained by direct uptake from water. This conclusion is supported by data collected on food chain uptake by freshwater fish (Williams and Pickering, 1961; Ball and Hooper, 1963).

The effect of increasing the environmental concentration of zinc-65 by a factor of two was to increase the initial rate of accumulation of the isotope by the fish. Joyner (1961) attributed the initial uptake of zinc-65 by brown bullhead, *Ictalurus nebulosus*, mainly to two sorption phenomena: (1) adsorption to mucus on surfaces in contact with the water, and (2) absorption by the transfer of zinc ions across semipermeable membranes of the fish. The results of the experiment reported in this paper indicate that doubling the amount of radioactive zinc in the water primarily affects these two pathways of accumulation, since it is the initial rate of accumulation that was affected. The rate of accumulation after 2 days was apparently similar in the two groups of fish.

LITERATURE CITED

BALL, ROBERT C., AND FRANK F. HOOPER. 1963. Translocation of phosphorus in a trout stream ecosystem. *In* Radioecology, Vincent Schultz and Alfred W. Klement, Jr. (eds.). Reinhold Publishing Corp., New York, and the American Institute of Biological Sciences, Washington, D. C., pp. 217–228.

BAPTIST, JOHN P., AND THOMAS J. PRICE. 1962. Accumulation and retention of cesium-137 by marine fishes. U. S. Fish and Wildl. Serv., Fish. Bull., 62: 177–187.

CHIPMAN, W. A., THEODORE R. RICE, AND THOMAS J. PRICE. 1958. Uptake and accumulation of radioactive zinc by marine plankton, fish and shellfish. U. S. Fish and Wildl. Serv., Fish. Bull., 58: 278–292.

DAVIS, J. J., AND R. F. FOSTER. 1958. Bioaccumulation of radioisotopes through aquatic food chains. Ecology, 39(3): 530–535.

DONALDSON, LAUREN R. 1959. Radiobiological studies at the Eniwetok Test site and adjacent areas of the western Pacific. U. S. Atomic Energy Commission, TID-5967, 18 pp.

JOYNER, TIMOTHY. 1961. Exchange of zinc with environmental solutions by the brown bullhead. Trans. Amer. Fish. Soc., 90: 444–448.

KRUMHOLZ, L. A. 1956. Observations on the fish populations of a lake contaminated by radioactive wastes. Bull. Amer. Mus. Nat. Hist., 110: 281–367.

LOWMAN, FRANK G., RALPH F. PALUMBO, AND DOROTHY J. SOUTH. 1957. The occurrence and distribution of radioactive non-fission products in plants and animals of the Pacific Proving Grounds. U. S. Atomic Energy Commission, UWFL-51, 61 pp.

NELSON, I. C. (Ed.). 1962. Evaluation of radiological conditions in the vicinity of Hanford for 1961. U. S. Atomic Energy Commission, HW-71999, 250 pp.

SCHIFFMAN, R. H. 1961. The uptake of strontium from diet and water by rainbow trout. U. S. Atomic Energy Commission, HW-72107.

WILLIAMS, LOUIS G., AND QUENTIN PICKERING. 1961. Direct and food-chain uptake of cesium-137 and strontium-85 in blue gill fingerlings. Ecology, 42(1): 205–206.